Vol. 100, No. 4, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 30, 1981 Pages 1453-1458

PERIODATE OXIDATION STUDIES IN THE ELUCIDATION OF THE STRUCTURES OF SIALIC ACID CONTAINING OLIGOSACCHARIDES

W.D. Gathmann & David Aminoff*

Departments of Internal Medicine (Simpson Memorial Institute)
and Biological Chemistry,
University of Michigan, Ann Arbor, MI 48109
U.S.A.

Received May 8, 1981

SUMMARY

The structures of sialo-oligosaccharide alditols as determined by ^{1}H-NMR spectrometry together with methylation analysis did not correspond with those derived previously from quantitative periodate oxidation data alone. Possible causes of the discrepancy were explored in the periodate oxidation methodology. No free sialic acid was released by the acidity of the periodate in the course of oxidation at pH 4.5. The anionic properties of the sialic acid residues were therefore utilized to separate the periodate oxidation products and thereby establish the position of the sialic acid in the oligosaccharide chain.

INTRODUCTION

Alkaline borohydride reductive cleavage (β-elimination reaction) is a useful method to release oligosaccharides from their linkage to serine and threonine in the polypeptide backbone. The presence of borohydride ensures the rapid conversion of the reducing end of the oligosaccharide chains released to the corresponding alditols, thereby preventing "peeling" of the oligosaccharide chains in the alkaline medium during the β-elimination reaction (1).

A comprehensive accounting of the oligosaccharides of porcine submaxillary mucin (2) was made by Carlson (3,4) using a pool of A^+ and A^-[§]

---

* To whom correspondence should be addressed.

§ Abbreviations used: A^+, A^- represent blood group A active and inactive glands, H^+ represents blood group H(O) active gland, A^-H^- glands with neither A or H activity and therefore inactive. Fuc, L-fucose; Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; GalNActitol, N-acetyl-D-galactosaminitol; NeuNAc, N-acetylneuraminic acid; NeuNGc, N-glycolylneuraminic acid; TBA, thiobarbituric acid.

0006-291X/81/121453-06$01.00/0

porcine submaxillary glands. A reinvestigation was undertaken by Aminoff and colleagues (5,6,7) in order to establish the role of the carbohydrate structure in determining the serological differences between the A^+, H^+ and A^-H^- phenotypically active glycoproteins (2). In the studies of both Carlson (3,4) and Aminoff et al (5-7), the structures of the isolated oligosaccharides were established utilizing techniques involving periodate oxidation by quantitation of the periodate consumed and the amount of formic acid and formaldehyde released.

A subsequent examination of the oligosaccharides by 360-MHz ^{1}H-NMR spectroscopy in combination with methylation and mass spectrometry was undertaken to provide alternative methods for the confirmation of the structures of the various isolated oligosaccharide alditols. The results (8,9) strongly suggest that in all three phenotypes A^+, H^+ and A^-H^-, the sialic acid is attached to N-acetylgalactosaminitol and not to galactose as previously proposed on the basis of results of periodate oxidation alone (5-7).

There was no disagreement with the structures for the neutral oligosaccharides. The discrepancy focused on the position of sialic acid on the oligosaccharide chain. Our attention was thus directed to the role that sialic acid might play in the interpretation of the periodate oxidation data.

Two aspects were examined in this study, namely a) whether sialic acid was released in the course of the oxidation at the acidic pH 4.5 and, if not, b) whether the anionic properties of the sialic acid residue could be utilized to separate the periodate oxidation products, thereby shedding light on the location of sialic acid on the oligosaccharide chain.

MATERIALS AND METHODS

Details of the source, serological typing and isolation of the glycoproteins from hog submaxillary glands have been published (5,6,7). The analytical methods and chromatographic procedures used in the isolation and characterization of the reduced oligosaccharides were also previously described (5,6,7).

TABLE 1
The composition and molar ratios* of carbohydrates in the oligosaccharide-alditols

Oligosaccharide-alditol	From Phenotype	Monosaccharide Residues (mol/mol of oligosaccharide) GalNActitol	Gal	Fuc	NeuNGc	GalNAc
Acidic-Di	A^+	0.97	0.00	0.00	1.00	0.00
	H^+	1.03	0.00	0.00	1.00	0.00
	A^-H^-	0.96	0.00	0.00	1.00	0.00
Acidic-Tri	A^+	1.02	1.03	0.00	1.00	0.00
	H^+	0.88	0.93	0.00	1.00	0.00
	A^-H^-	0.97	0.98	0.00	1.00	0.00
Acidic-Tetra	A^+	0.96	1.00	0.95	1.00	0.00
	H^+	0.96	1.07	0.99	1.00	0.00
	A^-H^-	1.03	1.07	0.99	1.00	0.00
Acidic-Penta	A^+	1.04	1.07	1.02	1.00	0.96

* The molar sugar composition of the acidic oligosaccharide alditols were calculated on the basis of one residue of NeuNGc.

PERIODATE OXIDATION

A) Release of Free Sialic Acid During Oxidation

Acidic trisaccharide-alditol, from A^+ hog submaxillary glycoprotein, was oxidized with periodate under the previously described conditions (5,6,7). The release of free sialic acid under the conditions utilized to follow the course of oxidation (5,6,7) was determined by the TBA assay (10).

B) Separation of Oxidation Products on Anion Exchange Resin

Oligosaccharide-alditols were treated with periodate at 4° using 0.1M sodium acetate buffer, pH 4.5 for 18 hr. After treatment with periodate and destruction of excess periodate by the addition of ethylene glycol, the oxidized oligosaccharides were separated into neutral and acidic components by passage through a small column (2 ml) of Bio-Rad AG-1-X2 (Cl^-, 200-400 mesh), followed by elution with 0.5 M NaCl to elute the anionic components from the resin. The percolate and eluate were then analyzed for their nitrogen content (11).

RESULTS AND DISCUSSION

Composition of the Oligosaccharides

The composition and molar ratios of carbohydrates present in the purified acidic oligosaccharide alditols are given in Table 1.

TABLE 2
Examination of periodate oxidation products for the presence of free sialic acid

Description	µmoles Total Sialic Acid	% Free Sialic Acid
NeuNGc	.05	100
NeuNAc	.05	100
Acidic Trisaccharide-alditol (A^+)	.58	0

Periodate Oxidation Studies and Release of Free Sialic Acid During Oxidation

Under the conditions of periodate oxidation used for the structural studies of oligosaccharide-alditols, both NeuNAc and NeuNGc remain fully reactive in the TBA assay for free sialic acid (10), Table 2. In contrast, there was no indication of the presence of free sialic acid, NeuNGc, in the course of oxidation of A^+ glycoprotein trisaccharide. Thus, it may be concluded that sialic acid is not released from the oligosaccharides under the conditions of oxidation with periodate at pH 4.5 for 18 hr.

TABLE 3
Analysis of acidic tetrasaccharides by periodate oxidation

Acidic tetrasaccharide-alditol	% Recovery of nitrogen H_2O Eluate	NaCl Eluate	Total
Expectation			
Fuc 1→2 Gal 1→3GalNActitol ↑(2,6) NeuNGc	0	100	100
Fuc 1→2 Gal 1→3GalNActitol ↑(2,6) NeuNGc	50	50	100
Experimental Findings			
A^+	51	50	101
H^+	46	47	93
A^-H^-	59	49	108

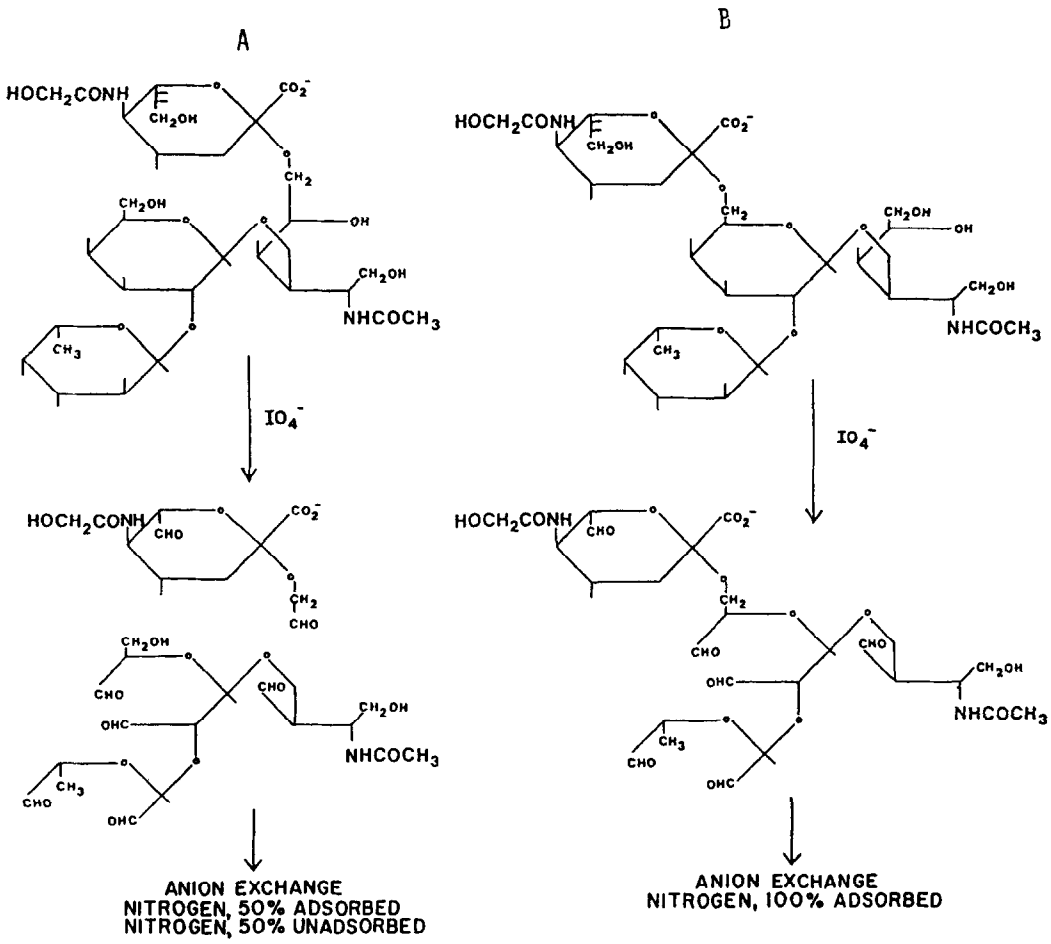

Figure 1. Periodate oxidation of acidic tetrasaccharide alditol and the expected products from A, where NeuNGc is attached to N-acetyl-galactosaminitol, and B, where NeuNGc is attached to galactose.

Position of Sialic Acid on the Oligosaccharide Chain

Periodate oxidation studies were carried on the acidic tetrasaccharides obtained from A^+, H^+ and A^-H^- hog submaxillary glycoproteins, and the oxidation products passed through an anion exchange resin. Table 3 shows the results obtained for the distribution of nitrogen, and indicates that the tetrasaccharides are cleaved by the periodate oxidation to yield a neutral and acidic nitrogen-containing component. The data are compatible with the sialic acid being attached to N-acetylgal-actosaminitol (Fig 1A) and not to galactose (Fig 1B). Moreover, the

results establish that there is no difference in the tetrasaccharide alditols derived from A^+, H^+ and A^-H^-. This finding is important, and confirms the observations previously made that there is no difference in the structure of oligosaccharides of H^+ and A^-H^- glycoproteins (7). The difference in their biological properties is attributable to the difference in secondary structures associated with the two phenotypes (7).

ACKNOWLEDGEMENTS

This investigation was supported by Grant HL AM 17881 from the National Institutes of Health.

REFERENCES

1. Neuberger, A., Gottschalk, A., Marshall, R.D., and Spiro, R.G. (1972) in: Glycoproteins their Composition, Structure and Function, Ed. Gottschalk, A., Elsevier Publishing Co., Amsterdam, pp 450-490
2. Aminoff, D., Morrow, M.P., and Zarafonetis, C.J.D. (1964) Fed. Proc., 23, 274
3. Carlson, D.M. (1966) J. Biol. Chem., 241, 2984-2986
4. Carlson, D.M. (1968) J. Biol. Chem., 243, 616-626
5. Baig, M.M., and Aminoff, D. (1972) J. Biol. Chem., 247, 6111-6118
6. Aminoff, D., Baig, M.M., and Gathmann, W.D. (1979) J. Biol. Chem., 254, 1788-1793
7. Aminoff, D., Gathmann, W.D., and Baig, M.M. (1979) J. Biol. Chem., 254, 8909-8913
8. Dorland, L., van Halbeek, H., Haverkamp, J., Vliegenthart, J.F.G., Fournet, B., Montreuil, J., and Aminoff, D. (1979) in: Proceedings of the Vth Int. Symp. on Glycoconjugates, Kiel, G.F.R., pp 29-30
9. van Halbeek, H., Dorland, L., Haverkamp, J., Veldink, G.A., Vliegenthart, J.F.G., Fournet, B., Ricart, G., Montreuil, J., Gathmann, W.D., and Aminoff, D. (1981) submitted for publication
10. Aminoff, D. (1961) Biochem. J., 81, 384-392
11. Schiffmann, G. (1966) Methods Enzymol., 8, 79-85